Supplementary information for Cronmiller et al. (2019) "Cell wall integrity signaling regulates cell wall-related gene expression in *Chlamydomonas reinhardtii",* submitted to Scientific Reports.

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**Supplementary Text S1.** Osmoregulation in *Chlamydomonas reinhardtii*

As *C. reinhardtii* is primarily a soil and freshwater-dwelling organism, tight osmoregulation is necessary for survival and to carry out cellular processes. This regulation is maintained by a number of mechanisms, most notably the pumping of water out of the cell via the contractile vacuoles (CVs) and the selective uptake of solutes from the environment. In higher plants, turgor pressure generated in the periplasmic space between the plasma membrane and cell wall is also known to contribute to osmoregulation. Whether turgor pressure plays a role in osmoregulation in *C. reinhardtii* is unclear, as early researchers assume yes (Hoffmann & Beck, 2005) and others assume no when considering the flexibility of the cell wall (Komsic-Buchmann et al., 2014). The model presented in Supplementary Figure S4a is an illustration demonstrating how *C. reinhardtii* cells are likely to maintain a suitable intracellular osmolarity. In a hypotonic environment, water will tend to move into the cell via osmosis. Anytime the intracellular water volume is too high (solute concentration is too low), the contractile vacuoles will pump the excess out of the cell. In a hypertonic environment, it is hypothesized that cells will pump solutes into the cell and/or accumulate solutes in the cytosol to avoid water loss, but direct evidence for this mechanism is lacking.

A measurement of the osmotic balance in these cells is known as the water potential – the tendency of water to enter or leave a cell – and can be understood as the sum of many pressure potentials. The pressures exerted on cells include: solute potential ($\Psi_s$), pressure potential ($\Psi_p$), gravimetric potential ($\Psi_g$), and matrix potential ($\Psi_m$) (Taiz et al., 2015). In an aquatic environment, gravimetric and matrix potentials are negligible, so pressure potential and solute potential are crucial to aquatic organisms (Taiz et al., 2015). It remains unclear how much of an effect the pressure potential has on *C. reinhardtii.* Certainly, the aqueous environment will exert a certain amount of hydrostatic pressure on the cell, but whether a turgor pressure is generated between the plasma membrane and cell wall is unknown. Given the specific water potential of a *C. reinhardtii* cell, the cell's surface area, and the permeability of water across the plasma membrane, one can generate an understanding about the rate of water movement into or out of the cell, called water flux (see equation in Supplementary Figure S4b).

A limited number of studies have been published about osmoregulation in *C. reinhardtii*. Existing research has focused primarily on the organization and mechanism of the CVs and their role in water efflux. Early studies by Luykx et al. (1997) demonstrated CV formation to be the dynamic amalgamation of scores of smaller water-containing vesicles, which eventually fuse with the plasma membrane as a large contractile vacuole and force water out of the cell. They also found several osmoregulatory mutants to have contractile vacuole defects, who's poor viability confirmed the importance of the CV in maintaining cytosolic osmolarity (Luykx et al., 1997b). More recent studies have found and characterized specific components of the CV system, such as the vesicular membrane protein SEC6 that facilitates CV membrane fusion and the aquaporin MIP1 which pumps water from the cytosol into the CV vesicles (Komsic-Buchmann et al., 2012; Komsic-Buchmann et al., 2014).

A study by Hoffmann & Beck (2005) considered the effects of cell wall removal on osmoregulation using three previously identified transcripts *GAS28, GAS30,* and *GAS31.* Their results indicated that in both hypo- and hyperosmotic media, cells will accumulate high levels of all three transcripts within two hours. However, this study may contain inaccuracies as the reported osmolarities for the test conditions and cytosol of *C. reinhardtii* differ greatly from the more recently calculated values presented in Komsic-Buchmann et al. (2014).

This limited research has focused almost solely on the cell's response to hypotonic environments and how cells actively remove excess water to maintain a water balance. We know very little about the cellular responses to hypertonic environments, aside from the cessation of contractile vacuole activity and decreased expression of *SEC6* and *MIP1* (Komsic-Buchmann et al., 2012, 2014). One study observed the intracellular accumulation of glycerol in cells exposed to a hypertonic environment (León and Galván, 1995). This response was presumed to prevent water loss in cells by raising the intracellular solute content.

**References**

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Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). *Plant physiology and development*. Sinauer Associates, Incorporated.

**Supplementary Table S1.** The CV period (contractile vacuole cycling times) in five different media of varying osmotic conditions. Data is presented in second. n = 3. NCV indicates no contractile vacuole cycle.

| Cell line | Media condition (osmolarity) | | | | |
|---|---|---|---|---|---|
| | $H_2O$ (0 mOsm) | ½ TAP (32 mOsm) | TAP (64 mOsm) | TAP+S (144 mOsm) | TAP+SS (204 mOsm) |
| CC-125 | 9.56 ± .40 | 10.90 ± .50 | 16.64 ± 1.12 | 22.91 ± 1.28 | NCV |
| CC-3491 | 17.66 ± 1.90 | 21.24 ± .73 | 27.16 ± 1.31 | 38.80 ± 1.65 | NCV |

**Supplementary Table S2.** *PHC19* promoter-driven luciferase activity of *cw15* progeny in response to g-lysin treatment. Luciferase activity is expressed as relative light units (RLUs) based on luminescence quantification. T1-1 and T1-3 did not have the *PHC19*-luciferase construct and were therefore observed only background level luciferase activity. Values are the mean of biological duplicate data.

| Tetrad | Luciferase activity (RLU) | | Genotype | |
|---|---|---|---|---|
| | - g-lysin | + g-lysin | *PHC19-gLUC* | *cw15* |
| T1-1 | 88 | 118 | no | mutant |
| T1-2 | 1998 | 1781 | yes | mutant |
| T1-3 | 61 | 128 | no | wild type |
| T1-4 | 197 | 956 | yes | wild type |

**Supplementary Table S3.** Candidate genes for putative cell surface sensors involved in the cell wall integrity signaling of *C. reinhardtii*. *annotation is based on V5.6 available at Phytozome.

| Gene_ID* | Name* | Comments | Amino acid length |
|---|---|---|---|
| ***TRP family group 1: With Pectin_lyase-like repeats (32 ea.)*** | | | |
| Cre01.g001300 | | HYP-rich | 5211 |
| Cre01.g040650 | | | 1201 |
| Cre01.g040850 | | | 2023 |
| Cre01.g042450 | | HYP-rich | 2673 |
| Cre01.g044350 | | Leu-binding domain | 1559 |
| Cre02.g094801 | | | 3739 |
| Cre02.g099055 | | | 4106 |
| Cre02.g118700 | | | 5013 |
| Cre03.g155700 | | | 2773 |
| Cre04.g219150 | | | 3718 |
| Cre04.g222650 | | HYP-rich | 3962 |
| Cre04.g227251 | | | 2251 |
| Cre06.g265652 | | | 4038 |
| Cre06.g305350 | | | 1389 |
| Cre06.g309650 | | | 3681 |
| Cre06.g309800 | MOT2 | | 5696 |
| Cre10.g419200 | | | 2898 |
| Cre10.g420600 | | | 1297 |
| Cre13.g564151 | | | 2404 |
| Cre13.g591300 | | HYP-rich | 5210 |
| Cre14.g619400 | | HYP-rich | 3196 |
| Cre17.g709000 | | HYP-rich | 4275 |
| Cre17.g728450 | | | 4007 |
| *Containing N-terminal Kelch domain* | | | |
| Cre01.g052750 | | HYP-rich | 4131 |
| Cre07.g338550 | | HYP-rich, EF-hand | 5889 |
| Cre13.g604050 | | HYP-rich, EF-hand | 3555 |
| Cre17.g706700 | | HYP-rich | 4056 |
| *Containing N-terminal SRCR domain* | | | |
| Cre04.g227500 | SRR18 | | 5536 |
| Cre04.g229550 | SRR15 | HYP-rich | 4138 |
| Cre10.g421900 | SRR27 | | 3737 |
| Cre12.g552700 | | HYP-rich | 4532 |

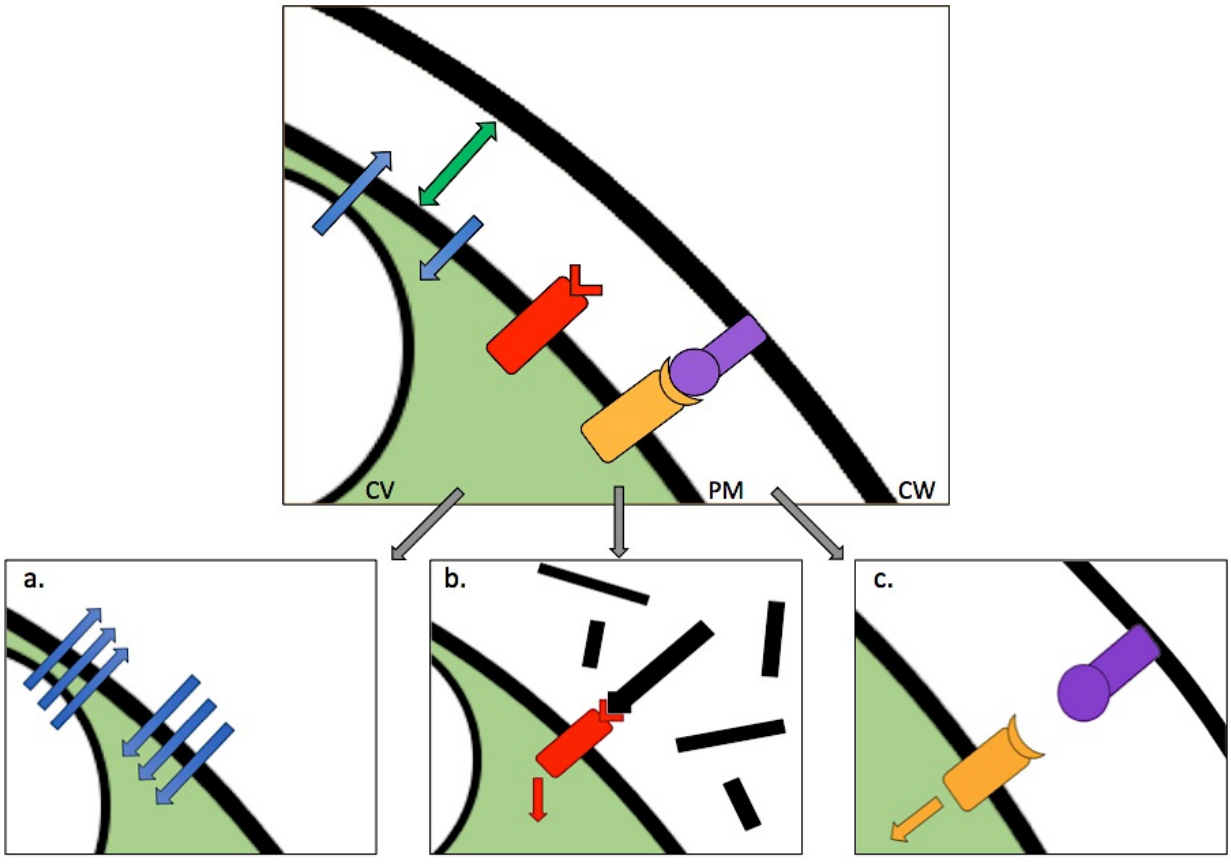
| | | | |
|---|---|---|---|
| Cre16.g654950 | SRR3 | | 4702 |
| ***TRP family group 2: PKD2-like (13 ea.)*** | | | |
| Cre03.g174100 | | HYP-rich | 4882 |
| Cre11.g475150 | | partial | 362 |
| Cre11.g479383 | | | 2673 |
| Cre12.g510350 | | HYP-rich, PAN domain | 6412 |
| Cre12.g515400 | | HYP-rich, DUF5011 domain | 3747 |
| Cre12.g539650 | | HYP-rich | 4829 |
| Cre13.g569550 | | | 4409 |
| Cre17.g715300 | PKD2 | | 1645 |
| *Containing lectin domains* | | | |
| Cre02.g115400 | | HYP-rich | 4211 |
| Cre06.g309951 | | HYP-rich | 7989 |
| Cre09.g400850 | CTL4 | HYP-rich, PAN/WSC domain | 8145 |
| Cre12.g515350 | | HYP-rich | 5155 |
| Cre16.g682550 | | HYP-rich, LRR domain | 7306 |
| ***Scavenger receptor cysteine-rich (SRCR) family (22 ea.)*** | | | |
| Cre03.g179450 | SRR7 | HYP-rich | 573 |
| Cre04.g219050 | SRR10 | HYP-rich, WSC domain | 2659 |
| Cre04.g227500 | SRR18 | TRP domain | 5536 |
| Cre04.g229550 | SRR15 | HYP-rich, TRP domain | 4138 |
| Cre08.g383400 | SRR11 | HYP-rich | 2127 |
| Cre08.g383600 | SRR21 | HYP-rich | 2097 |
| Cre09.g404750 | SRR2 | HYP-rich | 990 |
| Cre10.g421900 | SRR27 | TRP domain | 3737 |
| Cre10.g423850 | SRR26 | HYP-rich | 779 |
| Cre12.g552700 | | HYP-rich, TRP domain | 4532 |
| Cre13.g591550 | SRR20 | HYP-rich | 1286 |
| Cre16.g654950 | SRR3 | TRP domain | 4702 |
| Cre17.g706050 | SRR17 | HYP-rich | 4813 |
| *Containing N-terminal Trypsin-like domain* | | | |
| Cre01.g042300 | SRR6 | HYP-rich | 1905 |
| Cre01.g042352 | | HYP-rich (3000 a.a.) | 4951 |
| Cre01.g042502 | | HYP-rich | 3851 |
| Cre01.g045601 | | | 1628 |
| *Containing C-lectin domains* | | | |
| Cre01.g002787 | SRR24A | HYP-rich, GH18 domain | 4347 |
| Cre05.g245352 | | HYP-rich, PAN domain | 5227 |
| Cre10.g431050 | SRR13 | HYP-rich, PAN domain | 1874 |
| Cre10.g458350 | SRR16 | HYP-rich, GH18 domain | 9052 |

| | | | |
|---|---|---|---|
| Cre14.g631100 | SRR29 | HYP-rich, WSC/LysM domain | 2403 |
| ***MscS-like family (6)*** | | | |
| Cre01.g035750 | MSC3 | | 706 |
| Cre01.g055432 | | HYP-rich | 1524 |
| Cre07.g329300 | MSC1 | Chloroplast-localized | 493 |
| Cre10.g453400 | MSC4 | HYP-rich, EF-hand | 2899 |
| Cre12.g505800 | MSC6 | EF-hand | 1240 |
| Cre13.g591100 | MSC5 | HYP-rich, EF-hand | 2362 |
| ***OSCA1 (DUF221) family (11)*** | | | |
| Cre08.g360400 | ERM1 | | 1370 |
| Cre08.g360500 | ERM2 | | 1235 |
| Cre08.g360564 | | | 1217 |
| Cre08.g360600 | ERM4 | | 1482 |
| Cre09.g399912 | | | 1549 |
| Cre10.g460150 | | | 1558 |
| Cre12.g532151 | ERM7 | | 2129 |
| Cre12.g560050 | ERM12 | HYP-rich | 3083 |
| Cre13.g574850 | ERM6 | | 2110 |
| Cre13.g579400 | ERM8 | | 2239 |
| Cre17.g738150 | ERM10 | | 1887 |

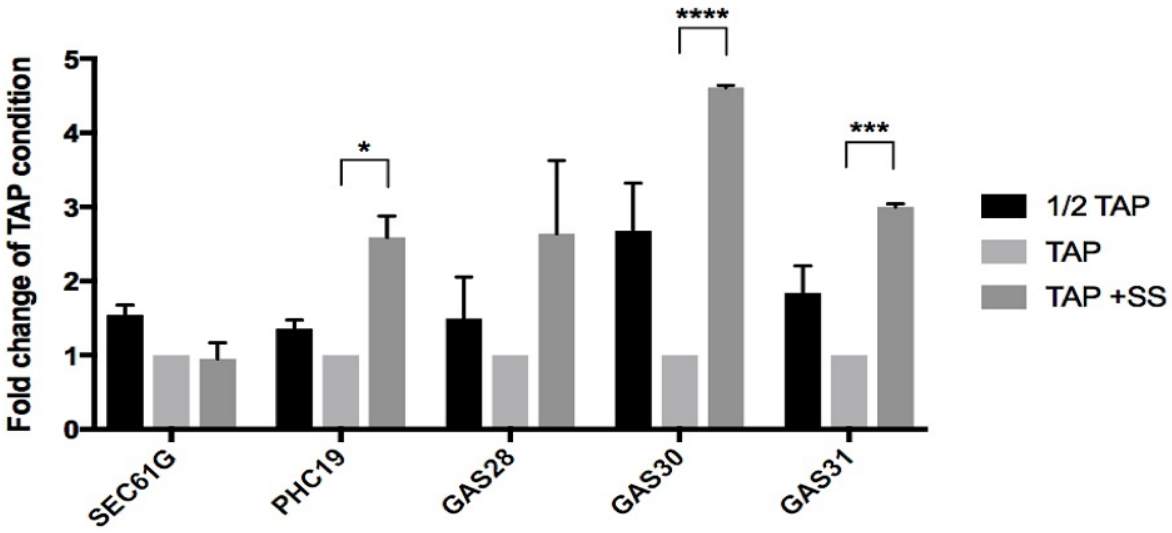
**Supplementary Table S4.** Primers used in this study

| Gene | Forward primer | Reverse primer |
|---|---|---|
| *RHM1* | ACGGGCTCAAGAAGACCATC | GTTTAGTTGGCCAGGAATGG |
| *AraGT1* | AGGTGCTGCACTTCACATCC | GTCTTGGTATCCCAGGTTCC |
| *SEC61G* | CACCAGCAACCATGGATC | CTTAGGAGCTCATGATGAC |
| *PHC19* | GCAGATATAGGGTGGGACGC | CCCTGGATCGTTCCCTTCTG |
| *GAS28* | AATCCATGCCGTACCAAGCA | CCTATGTGCGGACCTAGCAG |
| *GAS30* | ATGCCCGCAACAGTTACAGA | GCCCGAGGTTCTTGCTACTT |
| *GAS31* | CTGGCCACCTTCCCTTACTG | ACGCCGGTGTTGTATTGAGT |
| *RACK1* | GCCACACCGAGTGGGTGTCGTGCG | CCTTGCCGCCCGAGGCGCACAGCG |

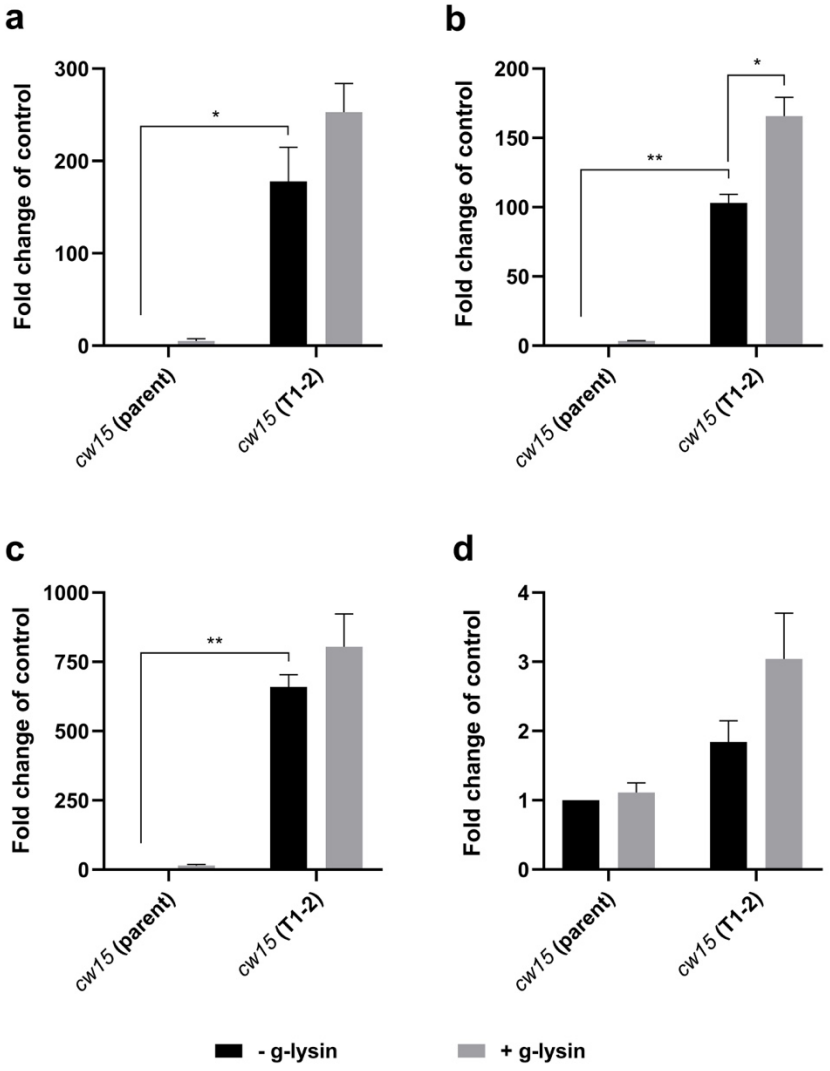
**Supplementary Figure S1.** Hypothetical signaling mechanisms for the g-lysin-induced responses. Illustrations represent a magnified region of the cell near the periphery. Top panel shows the possible elements responding to g-lysin treatment in an inactive (before treatment) condition including: receptor proteins as yellow and red shapes; water flow via contractile vacuole as blue arrows; hydrostatic pressure generated in periplasmic space as green double-headed arrow. Cellular elements are arranged spatially as denoted by CV, contractile vacuole; PM, plasma membrane; CW, cell wall. Bottom panels show active conditions by which cellular elements are stimulated and drive the g-lysin-induced response: (**a**) increased water flow into and out of the cell (increased flux rate); (**b**) receptor-mediated detection of fragmented/digested wall components; (**c**) receptor-mediated detection of physical wall removal.



**Supplementary Figure S2.** Transcript expression suggests modest cellular response to changing osmotic environment. Bar graphs represent the change in transcript activity in half-diluted liquid media (1/2 TAP, black bars) and liquid media plus sucrose (TAP +SS, medium grey bars) compared to the normal TAP media (light grey bars). mRNA levels are quantified as fold change of the TAP condition. Error bars represent one standard deviation from the mean of biological duplicate samples. Welch's t-test indicates statistical significance at $p \leq 0.05$ (*), $p \leq 0.001$ (***), $p \leq 0.0001$ (****); $\alpha = 0.05$.



**Supplementary Figure S3**. *cw15* progeny showed constitutive CW gene expression. (**a**-**d**) Bar graphs represent the change in gene expression for *PHC19* (a), *GAS28* (b), *GAS30* (c), and *SEC61G* (d) in *cw15* cells. Untreated control samples are represented by black bars, grey bars represent cells treated with g-lysin. Gene expression is quantified in terms of fold change compared to the untreated *cw15* samples. Error bars represent one standard deviation from the mean of biological triplicate samples. Welch's t-test indicates statistical significance at $p \leq 0.05$ (*), $p \leq 0.01$ (**); $\alpha = 0.05$.



**Supplementary Figure S4.** Diagrammatic representation of osmoregulation in *C. reinhardtii* cells. (**a)** 2D visualization of water flow through cells near the periphery. Important cellular structures labelled: CV, contractile vacuole; PS, periplasmic space; PM, plasma membrane; CW, cell wall; ECS, extracellular space. Water flux in/out of cell represented by blue arrows. Pressure potentials represented by orange arrows; $\psi_{Tp}$, turgor pressure; $\psi_{Hp}$, hydrostatic pressure. Solute transporter represented by pink shape and arrow. (**b**) Equation and derivation for total cellular water flux: A, surface area ($m^2$); $P_f$, permeability coefficient (m/s); $\psi_w$, water potential (Pa); $\psi_s$, solute potential (Pa); $\psi_p$, pressure potential (Pa); $\psi_g$, gravitational potential (Pa); $\psi_m$, matrix potential (Pa).